G.Gopi et al /J. Pharm. Sci. & Res. Vol.4(1), 2012,1676-1680

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Pharmacokinetic Evaluation of Metformin Hyrochloride with Stevias in Human Volunteers

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**Abstract**

The intestinal absorption of oral-anti diabetic drugs in the treatment of type-II diabetes mellitus is altered when they are concomitantly administered with synthetic drugs, food supplements and others. Diabetic health care consumers needs sweetening agents to take drugs, foods and eatables. A randomized cross over study in two phases and a washout period of 4 weeks was carried out to evaluate the bioavailability of anti diabetic drug Metformin hydrochloride when used with stevias a drug for ulcer. The study has been approved by the institutional ethical committee of Raja Muthiah Medical College & Hospital, Annamalai University. In the present study 10 healthy human volunteers received stevias (1g) for 5 days. After overnight fasting on 6$^{th}$ day a single dose of Metformin hydrochloride (500mg) was given. The blood samples following the intake were taken at different time intervals of 1, 2, 3, 4, 5, 7, 9 and 12 hours. The plasma samples (100µl) were injected into HPLC system after separation. The mobile phase comprised of Methanol: acetonitrile: mixed phosphate buffer (pH 2.6) at a ratio of (40:12:48). Analyses were run using cyano column (7.5mm x 4.6mm i.d, 5µm) at a flow rate of 1.2 ml.min$^{-1}$ with diode array detector operating at a detection wave length of 234 nm in HPLC and the pharmacokinetic parameters were calculated by using the software *Kinetica* (Version 4.4.1Innaphase, USA). This study reveals that there is no significant change in the plasma concentration of Metformin hydrochloride when it was concomitantly administered with Stevias.

**Keywords:** Bioavailability, Anti - diabetic drugs, Metformin hydrochloride, Stevias, Pharmacokinetics, Concomitant administration, Drug interaction.

## 1. INTRODUCTION

The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or (WHO/NCD/NCS/99.2) [1]. Currently diabetic mellitus is a great threat to the world community with more than 100 million persons suffering from diabetes. The prevalence and incidence of diabetes is increasing in most populations, being more prominent in countries like USA (< 16 million), Republic of China (< 14 million) and in Africa (< 20 million). India leads the world largest number of diabetic subjects and is being termed as "diabetes capital of the world", with 40.9 million people currently suffering from diabetes and expected to rise 69.9 million by 2025 [2]. Chronic elevation of blood glucose levels after onset of diabetes leads to many co-existing complications like diabetic retinopathy, diabetic neuropathy, peptic ulcer, diabetic foot ulcer and others. Drug therapy in type II diabetes becomes more complex as many individuals are on multiple drug therapy and administer many drugs during the same period of time to treat secondary diabetic complications [3]. Nagger and co-workers carried out a *in vitro* evaluation of antidiabetic drugs Metformin hydrochloride, Glibenclamide, Acetohexamide, Tolbutamide, Carbutamide, Tolazamide and Glymidine with various antacids and adsorbents such as magnesium trisilicate, aluminum hydroxide, calcium carbonate, magnesium oxide, talc, kaolin as well as charcoal and the authors reported that the antidiabetic drugs are strongly interacting with antacids/adsorbents in various degrees. The author's recommended to confirm such drug interactions *in vivo* [4]. In addition to these, an increasing number of drugs related complications arise day to day due to drug interactions [5-9]. Hence, a closer monitoring and supervision of multiple drug therapy is required for diabetes so as to avoid drug related complications and also Diabetic health care consumers needs sweating agents to take food, drugs, eatables and others to mask the undesirable taste in mouth particularly during critical care. Sweetening agents are available as synthetic and natural sources. Concomitant administration

of antidiabetic drugs with sweetening agents alters the pharmacokinetics of anti diabetic drugs due to erratic absorption. Recently, the clinician and clinical pharmacologist focusing their attention to address these complications for the better management of type II diabetes mellitus. The aim of the present study was to evaluate the bioavailability of Metformin hydrochloride when it concomitantly administered with a natural sweetening agents, Stevias. since Metformin hydrochloride is a biguanide, used in the treatment of type II diabetes [10].

## 2. MATERIALS AND METHODS

### 2.1 Materials

The base line HPLC studies were carried out using the pure sample of Metformin hydrochloride, received as a gift sample from Apex Laboratories, (P) Ltd, Chennai, India. The Metformin hydrochloride as a 500 mg tablets (GLICIPHAGE), Franco-Indian, Chennai and Stevia as a 1 g powder (STEVIAS), Procarvit Food Products India (Pvt Ltd.,) Coimbatore were used in the study. HPLC grade Acetonitrile and Analytical grade Acetic acid, Perchloric acid [11, 12] used for the study were received from Sd fine chemicals, Mumbai. Freshly prepared double distilled, deionized water, filtered through 0.2µm nylon filter (47 mm) using Millipore unit (USA), was used throughout the experiments [8]. The drug analysis was carried out using HPLC system (Shimaddzu LC -10 AD) having gradient pump (LC 10 AD UP) Rheodyne injector port and Photodiodearrya detector (SPD 10A VP). The data interpretation was done with Shimadzu system controller (SCL – 10 AVP).

### 2.2 Ethical Clearance for the Study

The ethical clearance for the present study was obtained by the proper representation and discussion of various ethical issues with human ethics committee of Raja Muthiah Medical College and Hospital, Annamalai University (Institutional Ethics Committee) with the number of M5/54/RMMC/04.

### 2.3 Subjects

Ten healthy subjects men age range from (21-30) weight range (57-79kg) participated in the study after obtaining a written informed consent and were ascertained to be healthy by medical history Clinical examination and routine laboratory tests. None even on medication. Study protocol was approved by ethics committee for studies in healthy subjects and primary care of the Rajah Muthiah Medical College & Hospital, Chidambaram.

### 2.4. Study Design

A randomized cross over study with two phases and a washout period of 4 weeks was carried out. Volunteers took 1 g powder (STEVIAS) orally once daily at 8 am for 5 days. After an overnight fast on the day $6^{th}$ at 8.00 am a single dose of Metformin hydrochloride as a 500 mg was administered orally with 150 ml of water [13-15]. Volunteers received a standard meal $3^{rd}$ hr after dosing and additional light standard meals at $7^{th}$ hr and $11^{th}$ hr after dosing [8].

## 3. EXPERIMENTAL

### 3.1 Pharmacokinetic evaluation of metformin hydrochloride

The pharmacokinetic evaluation of Metformin hydrochloride was carried out as per the method described by Yuen and co-workers [11] using HPLC system (UFLC Shimadzu Prominence LC - 20 AD) consisted of having isocratic pump (LC 20 AD UP), Rheodyne injector port and SPD M20A Shimadzu prominence diode array detector. The data interpretation was done with inbuilt Shimadzu system controller (SCL – 20 AVP). 5 ml of blood samples were withdrawn after oral administration of Metformin hydrochloride as a 500 mg tablet. The blood samples were withdrawn at various time intervals such as 1, 2, 3, 4, 5, 7, 9 and 12 hr and transferred into EDTA treated vacationer tubes and centrifuged at 5000 rpm for 10 min, the plasma separated and stored at 20°C until the analysis. The deproteinisation of the plasma was carried out by the treatment with perchloric acid at a ratio of 1:1, mixed thoroughly by vortex for 5 min and followed by centrification at 10,000 rpm for 10 min. The concentration of Metformin hydrochloride was estimated by injecting 100 µl of deproteinised supernatant liquid into HPLC using the mobile phase comprised of 0.01M potassium dihydrogen orthophosphate (pH 3.5) and acetonitrile at a ratio of 60:40 v/v, respectively.

### 3.2 Pharmacokinetic analysis

The drug concentrations of Metformin hydrochloride and Stevias were determined with the comparison of standard chromatograms. All the pharmacokinetic and statistical analysis were carried out by the interpretation of our data using the software *Kinetica* (Version 4.4.1, Innaphase, USA) [8] and the following parameters such as Peak plasma concentration ($C_{max}$), Time to $C_{max}$ ($T_{max}$), AUC, $t_{½}$ were calculated.

## 4. RESULTS AND DISCUSSION

The present study was conducted to assess the pharmacokinetics of Metformin hydrochloride when it was administrated with a natural sweetening agent Stevias. A randomized, two

cross over study with wash out period of 4 weeks are carried out. Volunteers took metformin hydrochloride (GLICIPHAGE 500 mg) once daily at 8 am for 5 days. After an overnight fasting on the day 6th at 8.00 am single dose of 1g of Stevias was administered orally with 150 ml of water. Blood samples were withdrawn before and after administration of Metformin hydrochloride. The plasma was separated and analyzed in HPLC system. The effect of Stevias on the pharmacokinetics of Metformin hydrochloride was also measured by concomitant administration of Stevias. The results of the study revealed that the pantoprazole does not alter the $C_{max}$, $T_{max}$, $t_{½}$ and AUC of Metformin hydrochloride (Fig 1&2). The study revealed that there was no alteration of pharmacokinetics parameters of Metformin hydrochloride when co administered with pantoprazole.

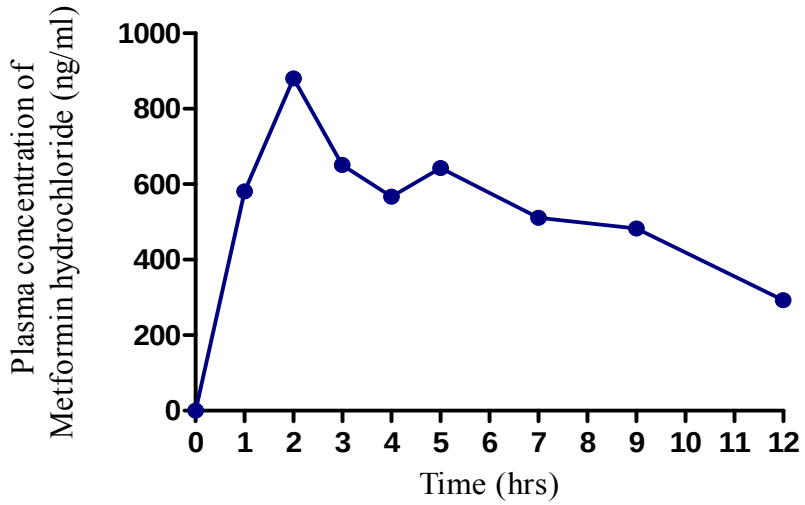


Fig 1: Plasma Concentration Time curve of Metformin hydrochloride after its oral administration (500 mg) in human volunteers.

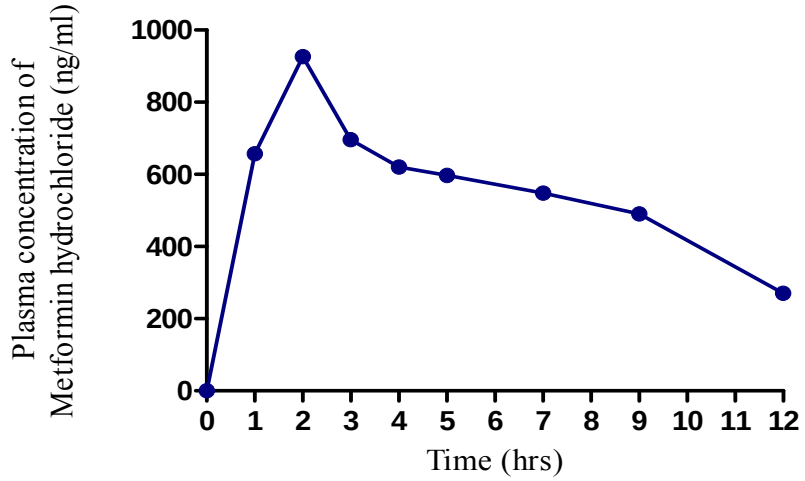


Fig 2: Plasma Concentration Time curve of Metformin hydrochloride after its oral administration (500 mg) with Stevias (1g) in pre treated human volunteers.

Table 1 Pharmacokinetic parameters of metformin hydrochloride in Stevias pretreated human volunteers

| Pharmacokinetics parameter | Metformin hydrochloride alone | Metformin hydrochloride with Stevias |
|---|---|---|
| $AUC_{0-t}$ (ng *h/mL) | 2734 ± 9.42 | 2652 ± 8.98 |
| $AUC_{0-\infty}$ (ng*h/mL) | 2748 ± 9.54 | 2693 ± 9.34 |
| $C_{max}$ (ng/mL) | 880 ± 8.35 | 927 ± 8.23 |
| $T_{max}$ (h) | 3.00 ± 0.58 | 3.00 ± 0.16 |
| $K_{el}$ (ng/mL) | 0.34 ± 0.03 | 0.26 ± 0.03 |
| $t_{1/2}$ (h) | 2.50 ± 0.39 | 2.55 ± 0.51 |

Volunteers took 500mg Metformin hydrochloride (GLYCIPHAGE) orally once daily for 5 days. After an overnight fast on the day 6 a single dose of 1 g powder of Stevias (STEVIAS) was administered orally.

## 5. CONCLUSION

The present study evaluated the effect of Stevias on the pharmacokinetics of Metformin hydrochloride and to investigate any possible interaction occur between metformin hydrochloride and Stevias. The drug concentrations of Metformin hydrochloride and pantoprazole were determined in comparison with standard HPLC chromatograms.

In conclusion, co administration of Stevias with Metformin hydrochloride does not change the pharmacokinetics of Metformin hydrochloride. It can be coadministered with metformin hydrochloride for the better management of type-II diabetes.

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